



# Biodiesel from wet microalgae: Extraction with hexane after the microwave-assisted transesterification of lipids



Jun Cheng\*, Rui Huang, Tao Li, Junhu Zhou, Kefa Cen

State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China

## HIGHLIGHTS

- Hexane extraction after microwave-assisted transesterification of lipids is proposed.
- FAME yield of hexane extraction from wet microalgae is increased 6-fold.
- FAME content in crude biodiesel is increased to 86.74% through hexane extraction.
- Polar pigments present in microalgae are not extracted into the crude biodiesel.

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## ABSTRACT

A chloroform-free novel process for the efficient production of biodiesel from wet microalgae is proposed. Crude biodiesel is produced through extraction with hexane after microwave-assisted transesterification (EHMT) of lipids in wet microalgae. Effects of different parameters, including reaction temperature, reaction time, methanol dosage, and catalyst dosage, on fatty acids methyl esters (FAMES) yield are investigated. The yield of FAME extracted into the hexane from the wet microalgae is increased 6-fold after the transesterification of lipids. The yield of FAME obtained through EHMT of lipids in wet microalgae is comparable to that obtained through direct transesterification of dried microalgae biomass with chloroform; however, FAME content in crude biodiesel obtained through EHMT is 86.74%, while that in crude biodiesel obtained through the chloroform-based process is 75.93%. EHMT ensures that polar pigments present in microalgae are not extracted into crude biodiesel, which leads to a 50% reduction in nitrogen content in crude biodiesel.

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## 1. Introduction

With growing energy shortage, alternate sources of energy, including biofuels, are receiving much attention. As sources of third generation biofuel, microalgae are considered as a promising alternative and a renewable feedstock source for biofuels. Microalgae are considered to be excellent candidates for fuel production because of their high photosynthetic efficiency, high growth rate, and high area-specific yield. Moreover, microalgae can be cultivated in saline/brackish water and on nonarable land; therefore, this precludes competition for the conventional crop land (Ananyev et al., 2008; Carrieri et al., 2008).

Traditional processes for biodiesel production from microalgae is complex and involves four steps, which are dewatering, grinding, oil extraction, and transesterification. To simplify the traditional process, Johnson and Wen (2009) reported a process that employs

chloroform and methanol as solvents and produces biodiesel through the direct transesterification of dried microalgae biomass under conventional heating. The yield of biodiesel has been shown to be 15% higher than that obtained when using the traditional extraction–transesterification process. However, when the chloroform-based direct transesterification process is employed to convert wet microalgae into biodiesel, the biodiesel yield is significantly lower than that obtained from the dried biomass.

Production of biodiesel from dried microalgae biomass involves the consumption of a large amount of energy for dewatering algae. To minimize the energy used for dewatering algae, several efforts have focused on developing methods for the production of biodiesel from wet microalgae. Lee et al. have reported that 80% of lipids in wet microalgae can be extracted by chloroform and methanol after the disruption of cell walls by microwave irradiation (Lee et al., 2010). Several organic solvents, including benzene, carbon tetrachloride, chloroform, n-hexane, and toluene, have been used in direct transesterification process to produce biodiesel from wet microalgae. While the process with chloroform has been

\* Corresponding author. Tel.: +86 571 87952889; fax: +86 571 87951616.

E-mail address: [juncheng@zju.edu.cn](mailto:juncheng@zju.edu.cn) (J. Cheng).

shown to result in high biodiesel yield (91%, based on the weight of lipid contained in the microalgae), the process with n-hexane as solvent has been shown to result in low biodiesel yield (33%; Im et al., 2014). When compared with the traditional two-step process using conventional heating, the production rate and yield of biodiesel is shown to be 6-fold and 1.3-fold higher when microwave irradiation is employed for direct transesterification of wet microalgae (Cheng et al., 2013b). However, reported processes that obtain high yields of biodiesels from wet microalgae involved the use of toxic chloroform, which is disadvantageous due to the safety concerns associated with its use.

Processes that avoid the use of chloroform and catalyst, involve the direct conversion of wet microalgae into biodiesel in the presence of supercritical alcohol (Patil et al., 2011b, 2013; Reddy et al., 2014). Although this process affords high yields of biodiesel (85%; based on the weight of lipid contained in the microalgae), stringent reaction conditions (high temperature and pressure) pose challenges for the efficient scale-up of the production. As free fatty acids have been shown to be more resistant to the effect of water on the production of fatty acids methyl ester (FAME) than triglycerides, Takisawa et al. have produced biodiesel by simultaneous hydrolysis–esterification of wet microalgal lipid (Takisawa et al., 2013a). After the reaction has been allowed to proceed for 6 h at 140 °C, a biodiesel yield of 90% (based on the weight of lipid contained in the microalgae) is obtained from wet microalgae, which contained 80% water. No chloroform has been used in this process, and the reaction temperature is much lower than that used in the supercritical alcohol process. However, the long reaction time of 6 h is needed to obtain the high yield of biodiesel. Microwave irradiation has been shown to effectively reduce the reaction time from 2 h to 10 min when dried microalgae biomass has been directly used to produce biodiesel (Ehimen et al., 2010; Wahlen et al., 2011). A microwave-assisted process using ethanol as reactant and hexane as solvent to produce biodiesel from dried microalgae biomass has also been reported by Martinez-Guerra et al. (2014). The yield of biodiesel from this process has been shown to be marginally higher than that from conventional Bligh and Dyer method. However, both the microwave-assisted processes have focused on the conversion of dried microalgae biomass; direct conversion of the harvested wet microalgae has not investigated.

To efficiently extract lipids from wet microalgae without using the toxic chloroform, a novel process, EHMT—extraction with hexane after microwave-assisted transesterification—is proposed in this paper. The effects of various parameters, including reaction temperature, methanol dosage, reaction time, and catalyst dosage, on the yield of FAME are investigated.

## 2. Methods

### 2.1. Materials

The microalgae (*Chlorella pyrenoidosa*) used in the experiments were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan. The microalgae were cultivated in Bristol's medium (Cheng et al., 2013a). The microalgae were cultivated in a 2 L bioreactor with an air pump for 15 d. Illumination (2500 lux) was supplied at the surface of the bioreactors with 12/12 h of dark/light cycle. The microalgae were harvested via centrifugation. The water content of the resulting paste was approximately 77 wt.%. Microalgae paste (~2 g) was collected in individual containers and stored at –20 °C until they were processed. Hexane, chloroform, methanol, and sulfuric acid were purchased from Sinopharm Chemical Reagent (China).

### 2.2. Biodiesel production through extraction with hexane after the microwave-assisted transesterification of lipids in wet microalgae

A WX-4000 microwave digestion system (2.45 GHz, Shanghai Yiyao Microwave Chemistry Company) equipped with six 60 mL digestion reactors was used to convert the lipids in wet microalgae. As shown in Fig. 1(a), wet microalgae biomass (~2 g), stored at –20 °C, was thawed at room temperature and transferred into the digestion reactor. Various dosages of methanol and sulfuric acid were subsequently added into the digestion reactor and mixed with the wet microalgae biomass. Then, the digestion reactor was sealed and heated via microwave irradiation to achieve the preset temperature. The microalgae biomass was maintained at the set temperature for different periods. The output of microwave oven was set at 600 W during the heating process and at 500 W during the holding process. After being subjected to microwaves, the mixture was allowed to cool to room temperature and transferred into a centrifuge tube. Reaction parameters, including reaction temperature (60–100 °C), ratio of volume of methanol-to-weight of wet microalgae biomass (12:1–4:1), reaction time (2–60 min), and catalyst concentration (1–5%), were varied to optimize the conditions for transesterification of lipids in wet microalgae.

Hexane (8 mL) was added to the mixture that was subjected to microwave-assisted transesterification. Extraction with hexane was performed by shaking the mixture vigorously for 5 min. The mixture was centrifuged and the upper hexane layer was transferred into a preweighed glass vial. The residue was shaken vigorously again in hexane to ensure that the biodiesel was completely extracted. The obtained hexane layer was transferred into the same glass vial and the hexane was evaporated in a baking oven at 65 °C. The mass of the crude biodiesel was gravimetrically determined.

### 2.3. Biodiesel production through transesterification after lipids extraction with hexane of wet microalgae pretreated with microwave-assisted disruption

As shown in Fig. 1(b), wet microalgae biomass (~2 g), stored at –20 °C, was thawed at room temperature and mixed with deionized water in the digestion reactor. Subsequently, the digestion reactor was sealed and subjected to microwaves till the temperature reached 90 °C. The aqueous solution of microalgae biomass was maintained at 90 °C for 30 min. The output of the microwave oven was set at 600 W during the heating process and 500 W during the holding process. After the treatment, the mixture was allowed to cool to room temperature and transferred into a centrifuge tube.

After the microwave-assisted disruption of wet microalgae (no transesterification before extraction), the mixture was extracted with hexane as described in Section 2.2. The transesterification of obtained lipids was further carried out at 90 °C for 30 min through microwave heating with 16 mL of methanol and 0.8 mL of sulfuric acid.

### 2.4. Biodiesel production through direct transesterification (simultaneous lipids extraction and transesterification) of dried microalgae with chloroform

As shown in Fig. 1(c), wet microalgae biomass (~2 g), stored at –20 °C, was thawed at room temperature and dried further in a baking oven at 65 °C. After grinding, the dried microalgae biomass was mixed with chloroform (8 mL), methanol (8 mL), and sulfuric acid (1 mL) in the digestion reactor. The digestion reactor was sealed and heated further in the baking oven at 90 °C for 1 h (Johnson and Wen, 2009). After the treatment, the mixture was allowed to cool to room temperature and transferred to a centrifuge tube. Distilled water (8 mL) was added to the centrifuge tube,

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